CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Oct. 2005, p. 1243–1245 Vol. 12, No. 10
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.10.1243–1245.2005

Immunogenicity of an Intranasally Administered Modified Live Canine Parvovirus Type 2b Vaccine in Pups with Maternally Derived Antibodies

Vito Martella,* Alessandra Cavalli, Nicola Decaro, Gabriella Elia, Costantina Desario, Marco Campolo, Giancarlo Bozzo, Elvira Tarsitano, and Canio Buonavoglia

Department of Animal Health and Well-Being, Faculty of Veterinary Medicine, University of Bari, S.p. per Casamassima km 3, 70010 Valenzano, Bari, Italy

Received 17 May 2005/Returned for modification 11 July 2005/Accepted 19 July 2005

The ability of a modified live canine parvovirus type 2b vaccine to elicit active immunization in pups with maternally derived antibodies (MDA) by intranasal administration was evaluated. The vaccine induced seroconversion in 100% of pups with MDA titers of ≤80 and in 51.6% of pups with titers between 160 and 320.

During the early 1970s, a new severe parvovirus-associated disease of pups was observed worldwide (1, 7, 20, 21). The novel parvovirus of canines (canine parvovirus type 2 [CPV-2]) most likely originated by a host species shift from feline parvovirus (FPV) or from FPV-like parvoviruses of wild carnivores. In the 1980s, two antigenic variants of CPV-2 (2a and 2b) arose almost simultaneously and within a few years completely replaced the original variant (6, 14, 15, 23, 26, 27, 28, 29, 34, 35, 36, 37, 38, 40). Intriguingly, only a few amino acid changes in the VP2 of FPV, CPV-2, and CPV-2a and -2b account for important antigenic/biological modifications (18, 25, 30, 39, 41). Additional mutations affecting important residues of the capsid protein VP2 of CPV (residues 297, 300, and 426) have been recognized recently, suggesting that CPV is still evolving (Table 1) (5, 19, 22, 24, 38).

Although the original variant of CPV-2 was completely replaced by the antigenic variants a few years after its appearance, the original CPV type 2 is still used in most commercial vaccines (4, 10, 11, 12). Altogether, there is concern that the antigenic differences between CPV type 2 and the CPV-2a and -2b variants may decrease the effectiveness of the CPV-2-based vaccines (16, 33, 42), and new modified live (ML) vaccines have been developed and licensed using CPV type 2b strains.

A major problem with the immunization of dogs against CPV is the persistence in pups of high levels of maternally derived antibodies (MDA) which may strongly interfere with the development of vaccine-induced immunity. Hemagglutination inhibition (HI) titers of ≥1:20 are able to interfere with an active immune response after vaccine administration, but such titers do not prevent infection with a virulent virus. In contrast, titers of ≥1:80 are considered fully protective against both infection and disease. With such MDA titers, equivalent to 2 to 4 maternal antibody half-lives (about 2 to 5 weeks), pups may fail to be successfully immunized and remain susceptible to infection (9, 12, 31).

* Corresponding author. Mailing address: Dipartimento di Sanità e Benessere Animale, Facoltà di Medicina Veterinaria, Università di Bari, S.p. per Casamassima km 3, 70010 Valenzano, Bari, Italy. Phone: 39 080 4679805. Fax: 39 080 4679043. E-mail: v.martella@veterinaria.uniba.it.

In previous studies, both the use of high-titer vaccines, given parenterally, and intranasal vaccination have been suggested as strategies to overcome the obstacle of MDA (Table 2) (2, 3, 8, 17). We are currently investigating whether the use of the new variant CPV-2b as a vaccine may enhance the efficacy of immunization against CPV infection. In a previous study (32), an ML CPV-2b vaccine with a relatively low virus titer administered parenterally proved to be highly effective in overcoming the obstacle of MDA (Table 2). The fact that a low-titer CPV-2b vaccine was successful in inducing active immune responses to CPV in pups with considerable levels of MDA directed our attention to evaluating the immunogenicity of the ML CPV-2b vaccine administered intranasally.

Seventy-eight 5- to 7-week-old pups were used from 16 litters born to bitches that had been vaccinated with a commercially available modified live CPV-2 vaccine before mating. The pups were immunized with a modified live CPV-2b vaccine (strain 29/97), attenuated by 68 passages on Crandell's feline kidney cells and with a titer of $10^{4.5}$ 50% tissue culture infectious doses ($TCID_{50}$) per ml (32). The vaccine was given by instilling 0.5 ml in each nostril. Pups were vaccinated initially at the age of 5 weeks. If they failed to seroconvert, they were revaccinated when they were 7 weeks old. Blood samples were taken and tested for antibodies to CPV by HI at the time of each vaccination and 15 days after the second dose. Serological responses were considered positive if antibody titers had increased at least threefold. The responses to intranasal vaccination are shown in Table 2. The geometric mean of the postvaccinal antibody titers in the pups that seroconverted was 3,564.71 (range, 640 to 20,480).

The main difficulty in controlling CPV infection in pups is due to interfering levels of MDA that can persist up to the age of 12 weeks, or longer, and that can suppress the development of active immune responses to vaccination. Other factors may affect the magnitude of the postvaccinal immune responses to CPV-2 vaccines, i.e., the vaccine virus titer, the degree of virus attenuation (i.e., serial passage level), the antigenic properties of the vaccine strain, and, importantly, the route of administration.

In this study, active immune responses after vaccination were observed in pups with high antibody titers (≤1:80) that

TABLE 1. Amino acid residues in the VP2 of parvoviruses of the feline subgroup

Virus	Origin, yr	Strain	Host	Protein at residue[a]:													
				80	87	93	101	232	265	297	300	305	323	426	555	564	568
FPV	USA, 1967	FPV-b	Cat	Lys	—	Lys	—	Val	—	—	—	—	Asp	—	—	Asn	Ala
MEV	USA, 1975	MEV-b	Mink	Lys	—	Lys	—	Val	—	—	—	—	Asp	—	—	Asn	Ala
CPV-2	USA, 1978	CPV-b	Dog	**Arg**	**Met**	**Asn**	**Ile**	**Ile**	**Thr**	**Ser**	**Ala**	**Asp**	**Asn**	**Asn**	**Val**	**Ser**	**Gly**
	USA, 1978	CPV-Norden	Dog	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccine A		Cornell 780916[b]	Dog	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccine B		154[b]	Dog	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CPV-2a	USA, 1984	CPV-15	Dog	—	Leu	—	Thr	—	—	—	Gly	Tyr	—	—	Ile	—	—
	USA, 1983	CPV-31	Dog	—	Leu	—	Thr	—	—	—	Gly	Tyr	—	—	Ile	—	—
CPV-2b	USA, 1984	CPV-39	Dog	—	Leu	—	Thr	—	—	—	Gly	Tyr	—	Asp	—	—	—
	USA, 1990	CPV-133	Dog	—	Leu	—	Thr	—	—	—	Gly	Tyr	—	Asp	—	—	—
Asp-300 CPV-2	Vietnam, 2000	LCPV-V203	Leopard	—	Leu	—	Thr	—	—	Ala	Asp	Tyr	—	Asp	—	—	—
	Vietnam, 2000	LCPV-V140	Leopard	—	Leu	—	Thr	—	—	Ala	Asp	Tyr	—	—	—	—	—
Pro-265 CPV-2	Italy, 2000	CPV-616	Dog	—	Leu	—	Thr	—	Pro	—	Gly	Tyr	—	Asp	—	—	—
	Italy, 2000	W42	Wolf	—	Leu	—	Thr	—	Pro	—	Gly	Tyr	—	Asp	—	—	—
Glu-426 CPV-2	Italy, 2000	136/00	Dog	—	Leu	—	Thr	—	—	Ala	Gly	Tyr	—	Glu	—	—	—
	Italy, 2000	56/00	Dog	—	Leu	—	Thr	—	—	Ala	Gly	Tyr	—	Glu	—	—	—
CPV-2b vaccine	Italy, 1997	29/97, 68th passage[b]	Dog	—	Leu	—	Thr	—	—	Ala	Gly	Tyr	—	Asp	—	—	—

[a] Dashes indicate identity to the sequence of the CPV-2 strain b (in boldface). CPV-2 variants of recent identification are also reported.
[b] These strains have been sequenced in this study.

were expected to block a high percentage of active immune pup responses. A possible explanation for these findings may be either an intrinsic strong immunogenicity of the vaccine used or the antigenic differences between CPV-2 and the variants CPV-2a and CPV-2b. In Table 1, the sequence of vaccine strain 29/97 (68th passage) is shown. Accordingly, the antigenic variants CPV-2a and -2b would be recognized partially by the antibodies raised to type 2 CPV (33), and MDA levels higher than previously estimated would be required to prevent the infection of pups by the CPV variants (9, 12, 13, 14). The interactions between CPV and the canine host were extensively studied in the 1970s and 1980s using exclusively the original CPV type 2. Therefore, the immunological parameters indicative of protection against the 2a and 2b variants of CPV are likely to be reconsidered (13).

Intranasal administration of the ML CPV-2b vaccine proved equally as effective as parenteral administration (Table 2), in spite of an additional 28 serial passages in tissue cultures.

TABLE 2. Comparison of the HI results of various experiments of vaccination with CPV-2 reported in the literature and the results of this study

HI titer	Strain and vaccine information or no. of pups with an active response/no. of pups vaccinated (%)[a]						
	Pratelli et al. (32)[b]: CPV-2b strain 29/97, 40th passage; parenteral; titer, 4.5 $TCID_{50}$	This paper[b]; CPV-2b strain 29/97, 68th passage; intranasal; titer, 4.5 $TCID_{50}$	Buonavoglia et al. (3)[b]; CPV-2 strain 17-80, ISS, >60th passage; parenteral; titer, 7.0 $TCID_{50}$	Buonavoglia et al. (2)[b]; CPV-2 strain 17-80, ISS, >60th passage; intranasal; titer 5.5 $TCID_{50}$	Carmichael et al (11); CPV-2 strain A, 100–115th passage; parenteral; titer, 5.5 $TCID_{50}$	Burtonboy et al. (8); CPV-2 strain NL-35-D, 37th passage; parenteral; titer, 7.0 $TCID_{50}$	Hoare et al. (17); CPV-2 strain NL-35-D, 13th passage; parenteral; titer, 7.0 $TCID_{50}$
<8						1/1 (100)	28/29 (96.5)
8						19/20 (95)	16/17 (94.1)
10	1/1 (100)	2/2 (100)	NT[c]	NT	(98)		
16						24/27 (89)	45/45 (100)
20	4/4 (100)	8/8 (100)	NT	NT	(50)		
32						18/22 (82)	31/33 (93.9)
40	12/12 (100)	20/20 (100)	72/78 (92.3)	14/14 (100)			
64						2/5 (40)	13/13 (100)
80	10/12 (83)	13/13 (100)		8/11 (72.7)	(0)		
128						2/3 (66)	2/3 (66)
160	4/7 (57)	10/15 (66.6)	0/128 (0)	3/17 (17.6)			
256						0/1 (0)	6/6 (100)
320	3/5 (60)	6/16 (37.5)		0/6 (0)			
640	NT	0/4 (0)		0/11 (0)			

[a] Differences in laboratory standardization may slightly affect the results reported in the various experiments.
[b] The results reported for these studies were obtained in the same laboratory.
[c] NT, not tested.

Whether the additional cultural passages may have affected the magnitude of the postvaccinal response is difficult to evaluate in this case. However, serial propagation in tissue cells has been shown to affect the vaccine's ability to overcome the MDA obstacle (Table 2) (8, 17). Also, these findings correlate with previous experiences in which immunization with an ML CPV-2 vaccine was shown to be equally as effective, or more so, when the vaccine was administered by the nasal route, though at a 30-fold-lower vaccinal dose (Table 2) (2, 3).

In conclusion, the findings of this study suggest that good protection against CPV infection may be achieved by the use of an ML CPV-2b vaccine administered intranasally. Active immunization of pups with low-titer ML CPV-2 vaccines has been shown to be affected even by negligible levels of MDA ($\leq$1:20) (Table 2), while our low-titer ML CPV-2b vaccine was able to induce active immune responses in all pups with MDA titers of $\leq$1:80. The findings of the present study reinforce the proposition that CPV-2b vaccines may confer more adequate protection than CPV-2 vaccines.

We are extremely grateful to Donato Narcisi and Filomena Cariola for their technical assistance and to Athina Papa for her editorial suggestions.

The work was supported by grants from CEGBA (Centro di Eccellenza di Genomica in campo Biomedico ed Agrario).

REFERENCES

1. **Appel, M. J. G., W. F. Scott, and L. E. Carmichael.** 1979. Isolation and immunization studies of canine parvo-like virus from dogs with haemorrhagic enteritis. Vet. Rec. **105:**156–159.
2. **Buonavoglia, C., A. Cavalli, E. Gravino, V. Voigt, D. Buonavoglia, and D. de Capraris.** 1994. Intranasal vaccination of pups with maternally derived antibodies with a modified live canine parvovirus. J. Vet. Med. B **41:**3–8.
3. **Buonavoglia, C., E. Tollis, D. Buonavoglia, and A. Puccini.** 1992. Response of pups with maternal derived antibody to modified-live canine parvovirus vaccine. Comp. Immunol. Microbiol. Infect. Dis. **15:**281–283.
4. **Buonavoglia, C., M. Compagnucci, and Z. Orfei.** 1983. Dog response to plaque variant of canine parvovirus. J. Vet. Med. B **30:**526–531.
5. **Buonavoglia, C., V. Martella, A. Pratelli, M. Tempesta, A. Cavalli, D. Buonavoglia, G. Bozzo, G. Elia, N. Decaro, and L. Carmichael.** 2001. Evidence for evolution of canine parvovirus type 2 in Italy. J. Gen. Virol. **82:**3021–3025.
6. **Buonavoglia, D., A. Cavalli, A. Pratelli, V. Martella, G. Greco, M. Tempesta, and C. Buonavoglia.** 2000. Antigenic analysis of canine parvovirus strains isolated in Italy. Microbiologica **23:**93–96.
7. **Burtonboy, G., F. Coignoul, P. P. Pastoret, and N. Delferriere.** 1979. Canine hemorrhagic enteritis detection of viral particles by electron microscopy. Arch. Virol. **61:**1–11.
8. **Burtonboy, S., P. Charlier, J. Hertoghs, M. Lobmann, A. Wiseman, and S. Woode.** 1991. Performance of high titre attenuated canine parvovirus vaccine in pups with maternally derived antibody. Vet. Rec. **128:**377–381.
9. **Carmichael, L. E.** 1983. Immunization strategies in puppies—why failures? 32nd Symposium on Small Animal Pediatrics. Comp. Cont. Educ. Pract. Vet. **5:**1043–1051.
10. **Carmichael, L. E., and L. N. Binn.** 1981. New enteric diseases in the dog. Adv. Vet. Sci. Comp. Med. **25:**1–37.
11. **Carmichael, L. E., J. C. Joubert, and R. V. H. Pollock.** 1983. A modified live canine parvovirus strain with novel plaque characteristics. I. Viral attenuation and dog response. Cornell Vet. **71:**408–427.
12. **Carmichael, L. E., J. C. Joubert, and R. V. H. Pollock.** 1983. A modified live canine parvovirus vaccine. II. Immune response. Cornell Vet. **73:**13–29.
13. **Decaro, N., M. Campolo, C. Desario, G. Elia, V. Martella, E. Lorusso, and C. Buonavoglia**. Maternally-derived antibodies in pups and protection from canine parvovirus infection. Biologicals, in press.
14. **De Ybanez, R. R., C. Vela, E. Cortes, I. Simarro, and J. I. Casal.** 1995. Identification of types of canine parvovirus circulating in Spain. Vet. Rec. **136:**174–175.
15. **Greenwood, N. M., W. S. K. Chalmers, W. Baxendale, and H. Thompson.** 1996. Comparison of isolates of canine parvovirus by monoclonal antibody and restriction enzyme analysis. Vet. Rec. **138:**495–496.
16. **Greenwood, N. M., W. S. K. Chalmers, W. Baxendale, and H. Thompson.** 1995. Comparison of isolates of canine parvovirus by restriction enzyme analysis, and vaccine efficacy against field strains. Vet. Rec. **136:**63–67.
17. **Hoare, C. M., P. DeBouck, and A. Wiesman.** 1997. Immunogenicity of a low-passage, high-titer modified live canine parvovirus vaccine in pups with maternally derived antibodies. Vaccine **15:**273–275.
18. **Hueffer, K., J. S. L. Parker, W. Weichert, R. E. Geisel, J.-Y. Sgro, and C. R. Parrish.** 2003. The natural host range shift and subsequent evolution of canine parvovirus resulted from virus-specific binding to the canine transferrin receptor. J. Virol. **77:**1718–1726.
19. **Ikeda, Y., M. Mochizuki, R. Naito, K. Nakamura, T. Myazawa, T. Mikami, and E. Takahashi.** 2000. Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. Virology **278:**13–19.
20. **Johnson, R. H., and P. B. Spradbrow.** 1979. Isolation from dogs with severe enteritis of a parvovirus related to feline panleukopaenia virus. Aust. Vet. J. **55:**151.
21. **Kelly, W. R.** 1978. An enteric disease of dogs resembling feline panleukopaenia virus. Aust. Vet. J. **54:**593.
22. **Martella, V., A. Cavalli, A. Pratelli, G. Bozzo, M. Camero, D. Buonavoglia, D. Narcisi, M. Tempesta, and C. Buonavoglia.** 2004. A canine parvovirus mutant is spreading in Italy. J. Clin. Microbiol. **42:**1333–1336.
23. **Mochizuki, M., R. Harasawa, and H. Nakatani.** 1993. Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan. Vet. Microbiol. **38:**1–10.
24. **Nakamura, M., Y. Tohya, T. Miyazawa, M. Mochizuki, H. T. Phung, N. H. Nguyen, L. M. Huynh, L. T. Nguyen, P. N. Nguyen, P. V. Nguyen, N. P. Nguyen, and H. Akashi.** 2004. A novel antigenic variant of canine parvovirus from a Vietnamese dog. Arch. Virol. **149:**2261–2269.
25. **Parker, J. S. L., W. J. Murphy, D. Wang, S. J. O'Brien, and C. R. Parrish.** 2001. Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. J. Virol. **75:**3896–3902.
26. **Parrish, C. R.** 1999. Host range relationships and the evolution of canine parvovirus. Vet. Microbiol. **69:**29–40.
27. **Parrish, C. R., C. F. Aquadro, M. L. Strassheim, J. F. Evermann, J.-Y. Sgro, and H. O. Mohammed.** 1991. Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. J. Virol. **65:**6544–6552.
28. **Parrish, C. R., P. H. O'Connell, J. F. Evermann, and L. E. Carmichael.** 1985. Natural variation of canine parvovirus. Science **230:**1046–1048.
29. **Pereira, C. A., T. A. Monezi, D. U. Mehnert, M. D'Angelo, and E. L. Durigon.** 2000. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. Vet. Microbiol. **75:**127–133.
30. **Pollock, R. V., and L. E. Carmichael.** 1983. Use of modified live feline panleukopenia virus vaccine to immunize dogs against canine parvovirus. Am. J. Vet. Res. **44:**169–175.
31. **Pollock, R. V. H., and L. E. Carmichael.** 1982. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. J. Am. Vet. Med. Assoc. **180:**37–42.
32. **Pratelli, A., A. Cavalli, G. Normanno, M. G. De Palma, G. Pastorelli, V. Martella, and C. Buonavoglia.** 2000. Immunization of pups with maternally derived antibodies to canine parvovirus (CPV) using a modified-live variant (CPV-2b). J. Vet. Med. B **47:**373–376.
33. **Pratelli, A., A. Cavalli, V. Martella, M. Tempesta, N. Decaro, L. E. Carmichael, and C. Buonavoglia.** 2001. Canine parvovirus (CPV) vaccination: comparison of neutralizing antibody responses in pups after inoculation with CPV2 or CPV2b modified live virus vaccine. Clin. Diagn. Lab. Immunol. **8:**612–615.
34. **Sagazio, P., M. Tempesta, D. Buonavoglia, F. Cirone, and C. Buonavoglia.** 1998. Antigenic characterization of canine parvovirus strains isolated in Italy. J. Virol. Methods **73:**197–200.
35. **Steinel, A., C. R. Parrish, M. E. Bloom, and U. Truyen.** 2001. Parvovirus infections in wild carnivores. J. Wildl. Dis. **37:**594–607.
36. **Steinel, A., E. H. Venter, M. Van Vuuren, C. R. Parrish, and U. Truyen.** 1998. Antigenic and genetic analysis of canine parvoviruses in southern Africa. Onderstepoort J. Vet. Res. **65:**239–242.
37. **Truyen, U., A. Gruenberg, S.-F. Chang, B. Obermaier, P. Veijalainen, and C. R. Parrish.** 1995. Evolution of the feline-subgroup parvoviruses and the control of canine host range in vivo. J. Virol. **69:**4702–4710.
38. **Truyen, U., A. Steinel, L. Bruckner, H. Lutz, and K. Mostl.** 2000. Distribution of antigen types of canine parvovirus in Switzerland, Austria and Germany. Schweiz. Arch. Tierheilkd. **142:**115–119.
39. **Truyen, U., and C. R. Parrish.** 1992. Canine and feline host ranges of canine parvovirus and feline panleukopenia virus: distinct host cell tropisms of each virus in vitro and in vivo. J. Virol. **66:**5399–5408.
40. **Truyen, U., G. Platzer, and C. R. Parrish.** 1996. Antigenic type distribution among canine parvoviruses in dogs and cats in Germany. Vet. Rec. **138:**365–366.
41. **Truyen, U., J. F. Evermann, E. Vieler, and C. R. Parrish.** 1996. Evolution of canine parvovirus involved loss and gain of feline host range. Virology **215:**186–189.
42. **Yule, T. D., M. B. Roth, K. Dreier, A. F. Johnson, M. Palmer-Densmore, K. Simmons, and R. Fanton.** 1997. Canine parvovirus vaccine elicits protection from the inflammatory and clinical consequences of the disease. Vaccine **15:**720–729.